CYTOGENETIC STUDIES ON TRIPLE TRANSLOCATION HETEROZYGOTES OF RICE

By

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The senoir writer previously reported double RT (short for reciprocal translocation) of chromosomes in rice obtained thronh the artificial synthesis from two single RT homozygotes. Seed-setting of the double RT heterozygotes was very poor (0—3%), though good pollen ratios varied most frequently from 20 to 30 percent. The fertility of their progenies was segregated into a ratio of 1:2:1, which was also true in the backcrossed F_1s of the double RT heterozygotes to the original variety, Taichung 65. In addition, extremely low percent of male gametes was found functional, despite 100 percent of functional female gametes detected in the double RT heterozygotes. He further conformed that one fourth of the female gametes of the double RT heterozygotes carried a normal chromosome set, another one fourth carried double RT and the remaining one half of the female gametes carried single translocation of either *a* or *b* in a equal frequency. However, he was not able to isolate any double RT homozygote due to the leathal effect of pollen carring double RT chromosome complement.

Several single RT homozygotes were treated with X-rays at a dose of 25,000^r (150 KVP, 20 mA, 0.1 *Al* filter, 20°C) and the semi-sterility segregating lines arosed from the irradiated material were screened for the new translocation that showed high frequency of ring of four chromosomes at diakinesis or metaphase I of meiosis. Through test-crossing of the fertile plants within such new RT lines to the original variety, the 2⊙4RT homozygotes were successfully isolated. These 2⊙4RT homozygotes were treated with r-rays from Co^{60} source again at a dose of 25,000^r and the semi-sterility segregating lines arosed from them were screentested for another new translocation as illustrated in Fig.1. Some cytogenetical observations on the 3⊙4 type of triple translocations of chromosomes will be reported here. The present work was started in 1962 and was stopped from 1963 for three years. It should be noted that the present report was financially supported in part by the National Council of Science Development to which the writers wish to show their gratitute. They are also grateful to the International Rice Research Institute for using its facilities in conducting a part of this work.

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MATERIALS AND METHODS

The rice variety used in this study was Taichung 65, which was, though old, still one of the leading varieties in Taiwan. The pedigree map, followed in this series of experiments including those reported previously, is shown in Fig.1. At the third time of irradiation, the dormant seed of one double RT homozygote of 2⊙4 type were treated with 25,000^{r} of r-rays, and the $3X_1$ generation was grown in a bulk plot, from which various semi-sterile panicles were selected. In $3X_2$ generation the panicle rows whose seed-fertility segregated into a ratio of 1:1 were screened for the RT showing high frequency of ring of four chromosomes. The fertile plants within these new translocation lines were test-crossed to the original variety, Taihung 65, to isolate triple RT homozygotes and to establish triple RT heterozygotes. The triple RT heterozygotes of 3⊙4 type were, thus, selfed and test-crossed to the original variety and their progenies were cytologically examined in comparison with their fertilities. The 1:3 aceto-alcohol was used to fix the young panicles at meiosis stage and the slides for cytological observation were prepared by the ordinary smear method.

RESULTS AND DISCUSSION

Double RT homozygotes have normal chromosome complement, excepting two exchanges of chromosomal segments between two pairs of chromosomes. Within double RT homozygotes which have been obtained in this study, no distinct difference in their plant characteristics from those of the original variety was found. This suggestes that position effect due to segmental interchanges is probably not common in rice.

Not all of the $3X_2$ panicle rows, of which each consisted of 15-20 individuals, showed the distinct 1:1 segregation ratio in seed-fertility. As shown in Table 1, however, some panicle rows had the seed-fertility segregated into the typical 1:1 ratio, even though the number of individuals within rows did not exceed 20.

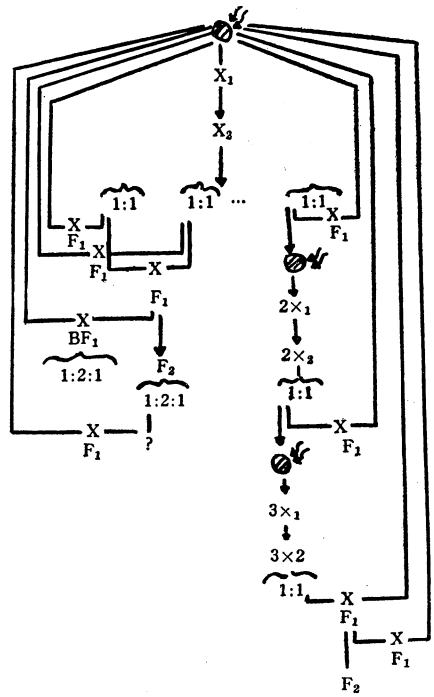

Fig. 1. The pedigree map involved in this series of studies, including those reported previously.

Table 1. X_2 generation of irradiated double RT homozygotes

Name of lines	Frequency of ring of four (%)	Number of plants	
		Fertile	Semisterile
RTH2⊙4X2—4	49.2	7	12
RTH2⊙4X2—7	83.4	2	10
RTH2⊙4X2—8	91.0	10	9
RTH2⊙4X2—10	79.0	6	12
RTH2⊙4X2—11	90.0	7	9
RTH2⊙4X2—14	100.0	3	7
RTH2⊙4X2—15	100.0	10	10
RTH2⊙4X2—18	82.7	10	9
RTH2⊙4X2—19	85.7	8	11
RTH2⊙4X2—22	91.6	3	10
RTH2⊙4X2—23	66.5	7	12

The frequency of ring of four chromosomes, observed at diakinesis or mataphase I of the semi-sterile plants within a line, varied from line to line. In some lines it even reached 100 percent. Segmental interchanges, occured in these lines, were particular in the chromosome structural background which was different from that of the original variety. According to the segmental interchange hypothesis, there should be two types of fertile plants within a new RT line, i.e., double RT homozygotes and triple RT homozygotes. This can be proved through the test-cross of these fertile plants to the original variety. The result, obtained, showed a fairly good fitness to the hypothesis. Seed-setting and good-pollen ratio of the triple RT heterozygotes which were established from the test-cross are shown in Table 2.

Good-pollen ratios of the triple RT heterozygotes varied from 1.86 to 6.22 percent. Accordingly, the theoretical good-pollen ratio can be calculated from the frequency of zigzag-shaped ring of four chromosomes. Since the overall average of zigzag ring was reported to be 0.478 in rice by Huang, *et al* (1958), the theoretical good-pollen ratio for the triple RT heterozygotes should be $(0.478)^3 = 0.109$. There existed considerable discrepancy between the observed and calculated values.

As for seed fertility, 0.87 to 1.56 percent were found most common in the plants grown in Taipei, while almost no seed-setting was observed in the stubbles of the same material grown in Los Banos, the Philippines. This difference in seed-setting might be attributed to ratoon culture that promoted the time of heading, consequently natural crossing could be greatly reduced. Generally speaking, the good-pollen ratio was greatly reduced due to the addition of one new translocation.

Table 2. Seed setting and pollen ratio of triple translocation heterozygotes

F_1 number	Parents ♀	Parents ♂	Type of chromosome pairing	In Taipei, Taiwan: Good pollen ratio (%)	In Taipei, Taiwan: Seed fertility (%)	Ratoon culture (in Los banos): O	Ratoon culture (in Los banos): △	Ratoon culture (in Los banos): ×
F_1 1150	Taichung 65	X_3RTH_2 III 1—10	3⊙4+6II	2.28	1.29	0	36.3	63.7
F_1 1155	Taichung 65	X_3RTH_2 III 3—2	3⊙4+6II	6.22	0.87	0	53.2	46.8
F_1 1156	Taichung 65	X_3RTH_2 III 3—2	3⊙4+6II	2.15	1.56	0	40.2	59.8
F_1 1157	Taichung 65	X_3RTH_2 III 3—3	3⊙4+6II	1.86	1.07	0	14.1	85.5
F_1 1158	Taichung 65	X_3RTH_2 III 3—4	3⊙4+6II	2.16	1.12	0	40.4	59.6
F_1 1159	Taichung 65	X_3RTH_2 III 3—7	3⊙4+6 II	3.33	1.34	0	32.7	67.3
F_1 1162	Taichung 65	X_3RTH_2 III 3—10	3⊙4+6II	2.76	1.26	0	67.1	32.9

O, % of filled grains

△, % of grains with parthenocarpic phenomenon

×, % of empty grains

Like those reported in the double RT heterozygotes (Huang *et al.* 1958) the rice grains showing parthenocarpic appearence were also observed in the triple RT heterozygotes. The percentage ranged most frequently from 30 to 50, which were about three times of those in the double RT heterozygotes.

The chromosome behaviour in meiosis of triple RT heterozygotes were thoroughly investigated, particularly at the stages of diakinesis and metaphase I. Since translocations in these triple RT heterozygotes, are independent of one another, the theoretical frequencies of all possible pairing types of chromosomes can be calculated from those determined when the three RTs were existed separately. If *a*, *b* and *c*, denote, respectively, the frequencies of ring of four chromosomes in translocation *a*, *b* and *c*, or *Ta*, *Tb* and *Tc*, thus, the frequencies of 12 II pairing type in *Ta*, *Tb* and *Tc* will be (1-a), (1-b) and (1-c), respectively. In a triple RT heterozygote, the theoretical frequencies of all possible pairing types of chromosomes in meiosis, therefore, can be generated by the following formula.

$$[a+(1-a)]\ [b+(1-b)]\ [c+(1-c)]$$

The values of *a*, *b* and *c* were previously determined to be 0.867, 0.957 and 0.895, respectively. The calculated and observed frequencies of all four possible types of chromosome pairing at diakinesis or metaphase I are shown in Table 3.

Table 3. Calculated and observed frequencies of pairng types of chromosomes in meiosis of triple RT heteroygotes

Pairing type	Formulation	Calculated %	Observed	
			(I)	(II) %
3⊙4+6II	abc	74.26	86	80.18
2⊙4+8II	ab (1-c)	23.44	26	14.21
	ac (1-b)			
	bc (1-a)			
1⊙4+10II	a (1-b) (1-c)	2.24	5	1.69
	b (1-a) (1-c)			
	c (1-a) (1-b)			
12II	(1-a) (1-b) (1-c)	0.06	0	0.14
Total		100.00	117	100.00

It is worthy to note that the secondary association of chromosomes in anaphase II was increased considerably and the number of chromosome association was also increased. This may be another approach to study the origen of so-called secondary association of chromosomes in rice.

As it would be expected, the triple RT homozygotes that were isolated from test-cross did not show distinct difference in morphological characteristics from the original variety, Taichung 65. However, whether they possess different quantitative characters remains unknown, since statistical treatment is necessary to answer this question.

Using triple RT heterozygotes as the female parent, backcrossing to the original variety was repeated for several seasons. The backcrossing, so far, has not been too successful, primarily due to the difficulty in getting open spikelets of the female parent for pollination, though hot-water and dark-room treatments were applied. Clipping method was also used, but did not give a satisfactory result. From a total of 855 spikelets pollinated, only 25 filled seeds were obtained. Among them, 14 individuals survived in the seed-bed nursery. The result did not provide a conclusive indication of what would be the rate of functional embryos in triple RT heterozygotes. The feeling is that the rate is not as high as that of fuctional embryos in the double RT heterozygotes, but neither as low as that of the functional pollens.

Good-pollen ratios, seed fertility and types of chromosome pairing of the 12 back-cross F_1 plants which were examined, are shown in Table 4. Among 12 backcross F_1 plants 5 plants were fertile, 2 plants were semisterile and the remaining 5 plants were almost completely sterile. The 5 fertile plants had the normal chromosome complement and another 5 plants, which were almost completely sterile, were identified to to be 2⊙4 RT heterozygotes through cytological observation. The remaining two semi-sterile plants did not show a clear-cut evidence of being single RT heterozygotes. It should be noted that no 3⊙4 RT heterozygotes was found.

Table 4. Good-pollen ratios, seed-fertility and chromosome pairing of the backcross F_1 plants of triple RT heterozygotes

Backcross number	Good pollen ratio	Seed fertility	Pairing type				
			3⊙4+6II	2⊙4+8II	1⊙4+10II	12II	Total
BF_1 (1)-1	47.9	24.2		3	17		20
BF_1 (1)-2	7.2	0.96	1	20	2		23
BF_1 (2)-2	94.5	93.9				20	20
BF_1 (2)-3	9.4	0.47		12	2		14
BF_1 (3)-1(1)	91.7	95.7				21	21
BF_1 (3)-1(2)	—	1.2		15	2		17
BF_1 (3)-2	—	92.9				20	20
BF_1 (5)-1(1)	96.2	85.3			1	22	23
BF_1 (5)-1(2)	—	23.7	2	9	12	2	25
BF_1 (6)-1(1)	93.7	93.7				20	20
BF_1 (6)-1(2)	—	0.97		19			19
BF_1 (6)-1(3)	—	4.5	3	8	8	6	25

If only the EMCs in which each of three rings of four chromosomes make alternate distribution at anaphase I survive, the theoretical frequencies of different female gametic types will be,

$(N+T)^3=1\ NNN+3\ NNT+3\ NTT+1\ TTT$

normal 1⊙4RT 2⊙4RT 3⊙4RT

Where *N* represents normal chromosomes, and *T* represents translocated chromosomes. Consequently, the backcross F_1s will segregate into the fertile, semi-sterile and almost completely sterile in a ratio of 1:3:4; remember 2⊙4RT and 3⊙4RT heterozygotes were found previously to have the similar seed-fertility.

The observed frequencies of different female gametic types diviated greatly from those expected from non-competition among different female gametes. For this reason, the diviation could be attributed to the competition among female gametes carring different chromosome strucutre, which was not found in double RT heterozygotes. Among 25 filled seeds of the backcross F_1, which germinated well, only 14 individuals survived. The unexpected low saviving rate was presumally due to anusually cold weather of the first rice crop of 1968, even though all hybrid material received careful protection in the green house. Whether this weather condition interacted with different female gametic types to cause such a diviation remains unknown, however, there was considerable indication of competition occurred among different female gametic types in the 3⊙4 RT heterozygotes.

From 3⊙4 RT heterozygotes, a number of seeds were collected. Out of 29 seeds that were collected from the material grwon in the green house, only 14 plants survived. In addition, 33 plants were secured from 76 seeds which were collected from the material grown in main fields The low surviving rate was probablly due to the anusually cold

weather of the first crop season of 1968.

The seed-fertilities of the progenies of several 3⊙4RT heterozygotes grown in the field condition as well as in the green house are shown in Tabel 5. Those of the backcross F_1s served for comparison.

Table 5. Seed-fertilities of the progenies of 3⊙4RT heterozygotes

Seed fertility (%)	2.5	7.5	15	25	35	45	55	65	75	85	95	Total
BF_1	5	—	—	2	—	—	—	—	—	1	4	12
F_2(G.H.)	9	—	—	3	—	—	—	1	1	—	—	14
F_2(Field)	23	2	3	4	1	—	—	—	—	—	—	33

The so-called F_2 in Table 5 could not be derived from self-fertilization, because of too low good pollen ratio of their parents (Table 2). The segregation of various plant characteristics in these so-called F_2 plants indicates that they were derived from natural cross-pollination. The segregation became more pronounced when the F_2s derived from the material grown in field condition were concerned. Apparently hybrid sterility interfered in the segregation of seed-fertility in the F_2s of Table 5.

No critical examination on the chromosome behavior at meiosis of these F_2 plants was made, however, the preliminary cytological observation on some of them indicates that chromosome pairing at meiosis was something complicated when compared with those observed in the backross F_1s.

The use of multiple interchange stocks for gamete selection by "Oenothera" method was suggested by Burnham (1946). For this, a multiple interchange stock is needed which produces a ring with all the chromosomes when crossed on a normal heterozygotes stock to be sampled and selected. Moreover, this big ring heterozygote should be able to produce the progenies having normal chromosome complement through self-fertilization. As mentioned before, neither double nor triple RT heterozygotes produced such normal progenies. Therefore, it is impossible to apply gamete selection, that was suggested by Burnham, to rice.

On the other hand, the present study demonstrated the feasibility of breeding the multiple interchange homozygotes through the consecutive irradiation of different levels of interchange homozygotes in rice. In other words, it is possible to develop a interchange homozygote or heterozygote of which translocations involve all chromosomes of rice. It will be of interest to see what will come out if the chromosmes of such a interchange heterozygote are doubled.

The 3⊙4RT homozygotes, obtained in the present study, differed from the original variety in chromosomal strucure by three reciprocal translocations of which evidence was the orientation of three rings of four at meiosis of their hybrids. If the 3⊙4RT homozygotes are crossed with the variety of other sub-species of *Oryza sativa*, minor structural difference of chromosomes, if exists, may interact with the three translocations and causes

some-more complicated pairing of chromosomes in the hybrids. The results of cytological observation on the F_2 plants, derived from open pollination in paddy field indicate such a possibility, however more critical examination is needed to verify this.

Summery

From the progenies of X-rayed single interchange homozygotes of a rice variety, Taichung 65, double interchange homozygotes were isolated. These homozygotes of two ring type were treated with r-rays and the triple interchange homozygote of three ring type were successfully isolated. Both double and triple interchange homozygotes did not show distinct difference in plant characteristics from the original variety.

The triple interchange heterozygotes, arosed from the test-cross, had good pollen ratios ranging from 2 to 6 percent and seed-fertility as low as 1.56 parcent or less. The seed-setting, though extremely low, was attributed to the outside pollen and a certain degree of functional embryos. Theoretical frequencies of pairing types of chromosomes in PMCs of triple translocation heterozygotes were compared with those frequencies observed. They showed a fairly good fitness.

Among 12 F_1 plants, derived from test-crossing of triple interchange heterozygote on the original variety, 5 were identified as double interchange heterozygotes, another 5 were normal in chromosomal strucutre and the remaining 2 were presumable to be single interchange heterozygotes or possibly double. There was considerable indication of competition occured among different types of female gametes in the triple interchange heterozygotes. The seed-fertility of the progenies of triple interchange heterozygotes was segregated in a different manner from that of the back cross F_1s. Apparently hybrid sterility interfered the segregation of the progenies which were derived from cross-pollination.

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稻三轉座異型體之細胞遺傳學的研究

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摘　　要

曾以X光線處理水稻品種臺中65號之單轉座同型體，並由其後代分離出雙轉座同型體，繼續以r-線處理此等雙轉座同型體，結果成功的分離出三轉座同型體。無論雙轉座或三轉座的同型體，其外表性狀均與原品種無多大差異。

三轉座異型體的正常花粉率甚低，約爲百分之2至6，結實率也只有百分之1.56或以下。極低頻度之結實率係由於天然雜交及有某程度的有效胚囊之關係。曾調查三轉座異型體於減數分裂時之染色體配對情形，並將之與理論者相對照。由三轉座異型體與原品種回交所得$F_1$12株中有5株爲雙轉座異型體，另5株爲正常，剩餘2株可能爲單轉座或可能雙轉座異型體，結果指出不同胚型之間很可能有競爭之現象。三轉座異型體後代之結實率分離比較複雜。因此等後代係由於天然雜交而來，很明顯雜種不稔性使此然之。

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